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The first gynandromorph of a zorapteran and potential thelytokous parthenogenesis in a population of *Zorotypus brasiliensis* Silvestri (Zoraptera: Zorotypidae)

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ABSTRACT

The first gynandromorph of the insect order Zoraptera is reported. A gynandromorph of *Zorotypus brasiliensis* Silvestri is described from a likely parthenogenetic population in the Atlantic Forest around the border of the Brazilian states of Minas Gerais, Rio de Janeiro, and São Paulo. As has been previously surmised for this species, the sample consisted of only females, tending to support the hypothesis of thelytokous parthenogenesis for this population. The gynandromorph is largely female in outward appearance, but exhibits male secondary sexual traits on the left side of the apical abdominal segments. The left (male) antenna consists of antennomeres of different proportions to those of the right (female) antenna. The internal genitalia and associated sclerites, however, are female although some are augmented in their form relative to normal-type females. Comments are made on possible factors resulting in the development of the gynandromorph. This is the 16th insect order with gynandromorphism reported.

Introduction

Gynandromorphism is the phenomenon by which an individual develops as a sexual mosaic of male and female traits. The distribution of male versus female attributes within the insect can be striking and clear, such as the expression of different sexual traits along a particular axis of the body, such as bilaterally asymmetrical individuals (*e.g.*, Wcislo et al., 2004; Michez et al., 2009; Hinojosa-Díaz et al., 2012). In the few systems where such a phenomenon has been investigated, they are the result of chimeric intermingling of genetically male and female tissues within a single individual (Narita et al., 2010). This may result from a variety of developmental aberrations or, in some hymenopteran species with *Wolbachia*-induced parthenogenesis, higher temperatures partially suppress the action of the bacterial symbiont and result in the formation of gynandromorphs (*e.g.*, Bowen and Stern, 1966; Cabello-García and Vargas-Piqueras, 1985). Aside from a few model systems, however, the developmental mechanisms of gynandromorphism have never been explored owing to the rarity of such individuals. Instead, the presence of gynandromorphs within most species for which they are known are simply reported and characterized (*e.g.*, Wcislo et al., 2004; Engel and Hinojosa-Díaz, 2011; Skvarla and Dowling, 2014; Spring et al., 2015), but their frequency or underlying genetic architecture remains obscure. Indeed, in the cases of those individuals reported as mixed gynandromorphs, whereby the distribution of male versus female traits are not clearly separated along some axis and are instead intermingled, it remains to be determined whether or not they are genetically intersexes rather than true gynandromorphs. Nonetheless, gynandromorphs have been reported in 15 Orders of insects with dramatically different underlying sex-determination systems, namely: Ephemeroptera, Odonata, Plecoptera, Orthoptera, Phasmatoe, Dermaptera, Blattaria, Hemiptera, Psocoptera, Coleoptera, Hymenoptera, Trichoptera, Lepidoptera, Diptera, Siphonaptera (Cui and Cai, 2003; Narita et al., 2010) and now Zoraptera, the 16th Order.

Gynandromorphs have the potential to inform us about the underlying sex determining mechanisms in certain insects (Narita et al., 2010), and can also be used to refine hypotheses of homology between sexually dimorphic structures such as the genitalia (*e.g.*, Michener, 1943; Engel, 2007).

Here we report the first gynandromorph for the insect order Zoraptera from a population of *Zorotypus brasiliensis* Silvestri

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sampled in the Brazilian Atlantic Forest near Londrina, Paraná. When describing the species, Silvestri (1947) hypothesized that the species was potentially parthenogenetic as when he reared individuals in the laboratory, he only obtained females. No subsequent observations have been reported for the species and no further investigation has been made into the biology or hypothesis of parthenogenesis. Although we were not able to rear individuals in a controlled setting, aside from the gynandromorph, all sampled individuals were females, tending to support Silvestri's conclusions.

Material and methods

Specimens were collected under the bark of rotting wood using an electrical aspirator, and then stored in 70% ethanol. The abdomen of certain individuals was removed and macerated at 100 °C in 85% lactic acid for approximately 15–60 min, and then dissected and photographed while in glycerin on a slide. The head and thorax were not dissected. The entire specimen, including the abdominal preparation, was ultimately preserved in glycerin within a microvial. The voucher of the gynandromorph is deposited in the *Instituto Nacional de Pesquisas da Amazônia* (INPA), Manaus; specimens of the normal females will be distributed among the following Brazilian institutions: INPA; *Museu Paraense Emílio Goeldi* (MPEG), Belém; *Museu de Zoologia da Universidade de São Paulo* (MZSP), São Paulo; and *Museu Nacional do Rio de Janeiro* (MNRJ), Rio de Janeiro.

Morphological terminology generally follows that of Rafael and Engel (2006) and Rafael et al. (2008), with terms for the female genitalia adapted from Delamare Deboutteville (1956) and Silvestri (1947). The terms alate, dealate, and apteron are used for individuals of the winged morph with fully developed compound eyes and ocelli still possessing their wings (alate), after shedding their wings (dealate), or the blind, wholly wingless morph (apteron) (Engel, 2008).

Photographs were taken through a Leica DFC500 digital camera fitted on a Leica MZ205 stereomicroscope connected to a computer with the Leica Application Suite software, which includes an Auto-Montage module (Syncroscopy software) which combines multiple layers of photographs into a single fully focused image. The apex of the abdomen was removed and macerated in 85% lactic acid heated at 100 °C for about 30 min. The macerated abdomen was examined on excavated slides with glycerin. The dissected terminalia were placed in microvials with glycerin. All specimens were stored in 80% ethanol.

Results

Zorotypus brasiliensis Silvestri, 1947
Gynandromorph (Figs. 1–3)

Description

Gynandromorph (left half male, right half female); apteron morph (*i.e.*, blind, wingless morph). As generally described for normal *Z. brasiliensis* except as follows: Body brown to dark brown; right antenna with nine antennomeres, left antenna with 7 antennomeres (apical most two flagellomeres lost during preparation), antennomeres 2–5 on male half reduced (likely not fully developed, with antennomeres prior to elongation and subdivision), antennomeres 1, 6, and 7 on male half longer than corresponding antennomeres of female half (Fig. 1a). Left metafemur (male half) with posteroventral row of ten stout, erect setae, gap between median series (four setae) and subapical series (five setae), those most subapical setae reclined apically (Fig. 1b). Right metafemur (female half) also with posteroventral row of ten setae with slightly different arrangement, with gap between median series (six setae) and subapical series (three setae). Abdominal terga each with uniserial posterior row of 12–14 setae, and generally 2–3 more anterior, smaller, posterolateral setae near border; tergum X, left of midline (male half), with two groups, anterior and posterior, of two thick, black sensilla basiconica (Fig. 2a and b) (male of *Z. weidneri* New and an undescribed species from Espírito Santo have three anterior and two posterior sensilla basiconica in males); right side (female half), with uniserial transverse row of elongate setae (Fig. 2b: as in normal-type females, *cf.* Fig. 2c and d). Epiproct somewhat squarish to subtrapezoidal, inconspicuously divided along midline proximally (Fig. 2b) (in males of *Z. weidneri* and the undescribed species from Espírito Santo the epiproct is entirely divided into hemitergites along the midline, while in females the halves are fused along the midline, implying that the form of the gynandromorph is intermingled between male and female traits, *i.e.*, partially fused), lateral portions of epiproct extending around cercal base; small mating hook (=anal sclerite) (present only in males) somewhat straight (rather than curved), directed upward, medially placed (Fig. 2a and b) (in males of zorapterans the mating hook is usually placed more medioposteriorly relative to tergum X; in males of *Z. weidneri*, the undescribed species from Espírito Santo, and most other Zoraptera the hook is distinctly upcurved distally). Paraproct somewhat thin, slightly sclerotized, extending from cercal base to midline, with 2–3 stout setae at medial margin. Sternum VIII (Fig. 3a) with shallow, broadly concave medial emargination (as in females), albeit entire sternum not as enlarged

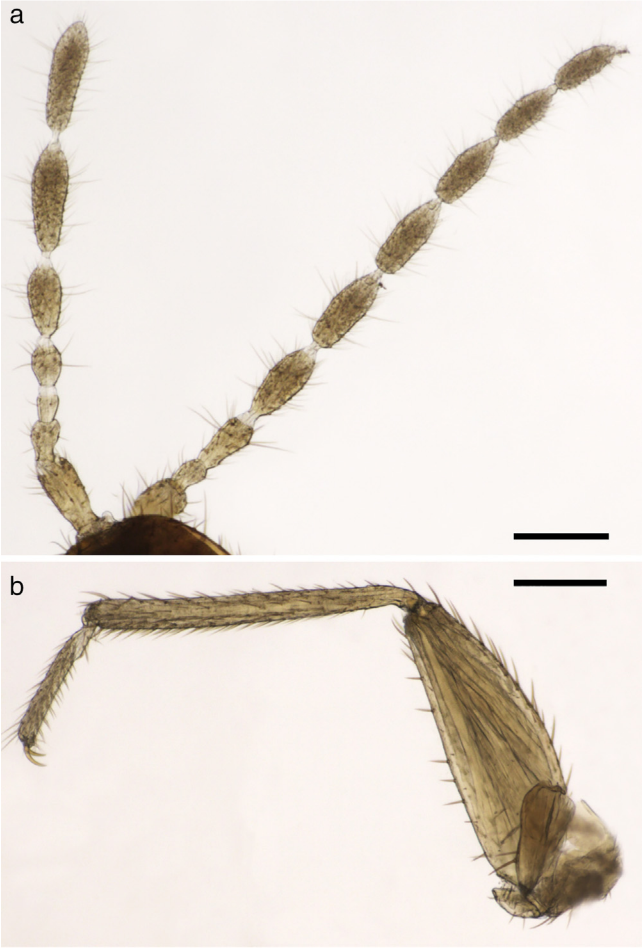

Fig. 1. Bilateral gynandromorph of *Zorotypus brasiliensis* Silvestri from near Londrina, Paraná, Brazil. (A) Antennae. (B) Left metafemur (male side). *Scale* = 0.2 mm.

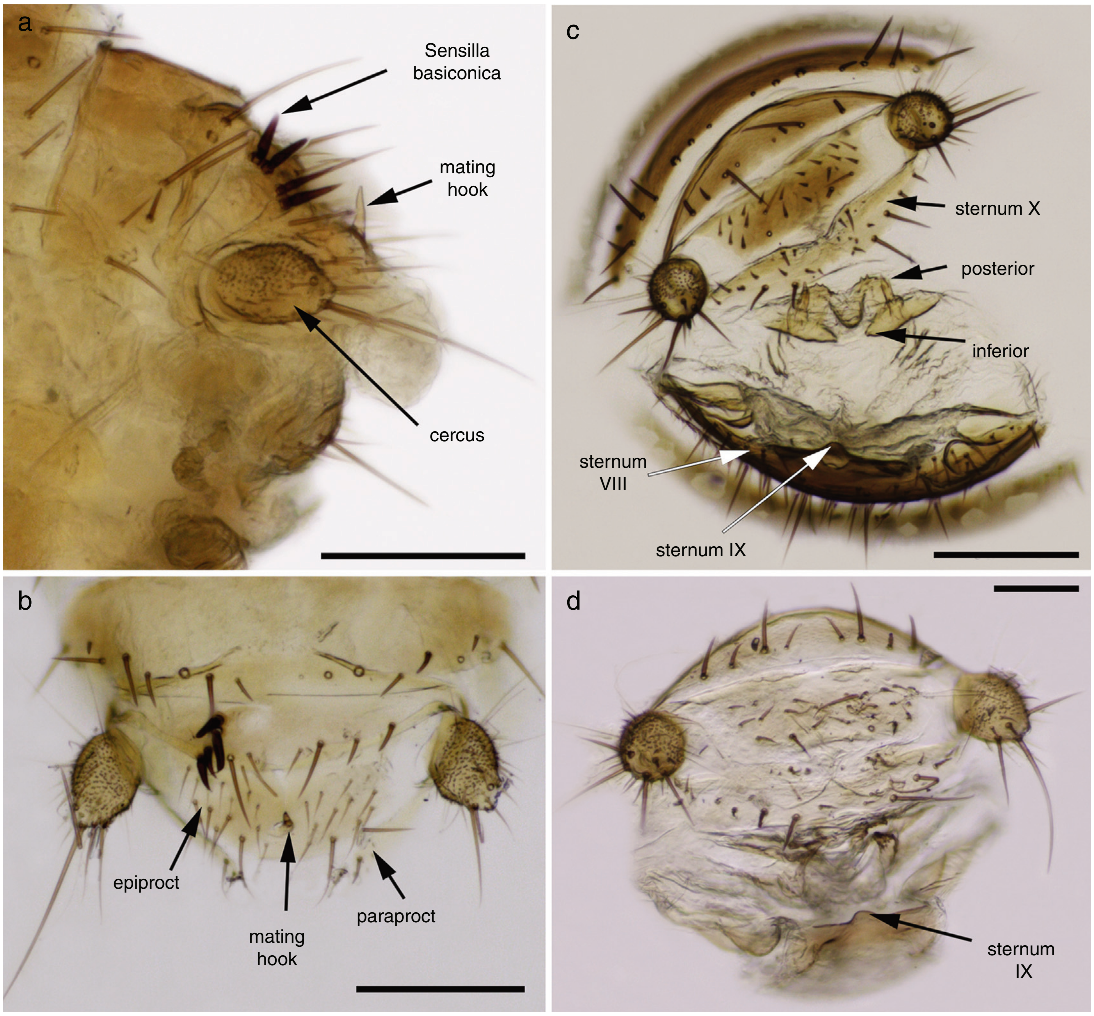

Fig. 2. Abdominal apex of gynandromorph and normal-type female of *Zorotypus brasiliensis* Silvestri. (A) Left lateral view of abdominal apex of gynandromorph depicting male traits typical of many species of *Zorotypus* (*i.e.*, mating hook and sets of sensilla basiconica). (B) Dorsal view of abdominal apex of gynandromorph (left half is male, right half is female). (C) Apical abdominal view of normal female. (D) Apical view of abdominal apex of normal female with sternum VIII removed to show remnant of sternum IX. *Scale* = 0.2 mm, except for *D* = 0.1 mm.

as in normal-type females. Sternum IX reduced, juxtaposed to sternum VIII, lightly sclerotized over much of disc (normal-type sternum IX in Figs. 2c, d and 3d), basally with broad, shallow medial concavity; apical margin slightly folded ventrally as thin lip to meet margin of sternum VIII, allowing for aperture of gonopore. Gonopore positioned between sternum VIII and IX. Sternum X (usually identified as sternum IX in earlier papers) of normal-type form (Silvestri, 1947: as sternum IX). Internal genital pouch (Fig. 3b and c) with typical arrangement of lobes in normal-type females (vide Silvestri, 1947), albeit somewhat degenerate: median, posterior, inferior, convergent, sublateral, and lateral lobes present (Fig. 3c). Spermatheca slightly pyriform (more ovoid in normal-type females), with long, thin duct extending to genital fork (vulva) (Fig. 3b).

Material examined

Gynandromorph apteron morph: Brasil, Paraná, Londrina, Parque Estadual Mata dos Godoi, 23°27′18″ S – 51°14′19″ W, 02.ii.2003, J.A. Rafael and J. Lopes, sob casca de árvore [underbark of rotting wood] (INPA).

Normal adult female specimens, all collected under bark of rotting wood and to be deposited at INPA, MPEG, MZSP, and MNRJ.: Four samples of 17♀♀ (15 apterons, 2 dealates), Brasil, Paraná, Londrina, Parque Estadual Mata dos Godoi, 23°27′18″ S – 51°14′19″ W, 02.ii.2003, J.A. Rafael and J. Lopes; 2♀♀ (apterons), Paraná, Terra Boa, Sitio Indaiá, 23.xii.2002, J.A.Rafael; three samples with 27♀♀ (25 apterons, 2 dealates), Paraná, Tijucas do Sul, 25°50′13″ S – 48°56′22″ W, 750 mts [Serra do Mar], 04.xii.2006, J.A.Rafael; five samples with 9♀♀ (7 apterons, 2 dealates), Santa Catarina, Itapoã, 26°05′32″ S – 48°38′23″ W, 16.i.2003, J.A.Rafael.

Discussion

Among the 55 specimens examined, five individuals were dealates while the remainder were apterons. The absence of males matches that observed by Silvestri (1947) and is peculiar for Zoraptera where males and females are usually easily found together, with even comparatively small samples typically recovering both sexes (e.g., Engel, 2002; Engel and Falin, 2008; Mashimo et al., 2013; Yin et al., 2015; Wang et al., 2016). Although continued sampling is certainly needed as well as rearing in the lab in order to more definitively confirm the natural absence of males, it would appear as though the populations known exhibit thelytokous parthenogenesis. Certainly, some of the collected females had well-developed eggs within the abdomen, further consistent with Silvestri's hypothesis of parthenogenesis relative to alternatives such as extreme sex-ratio bias. Whether this is the result of infection by

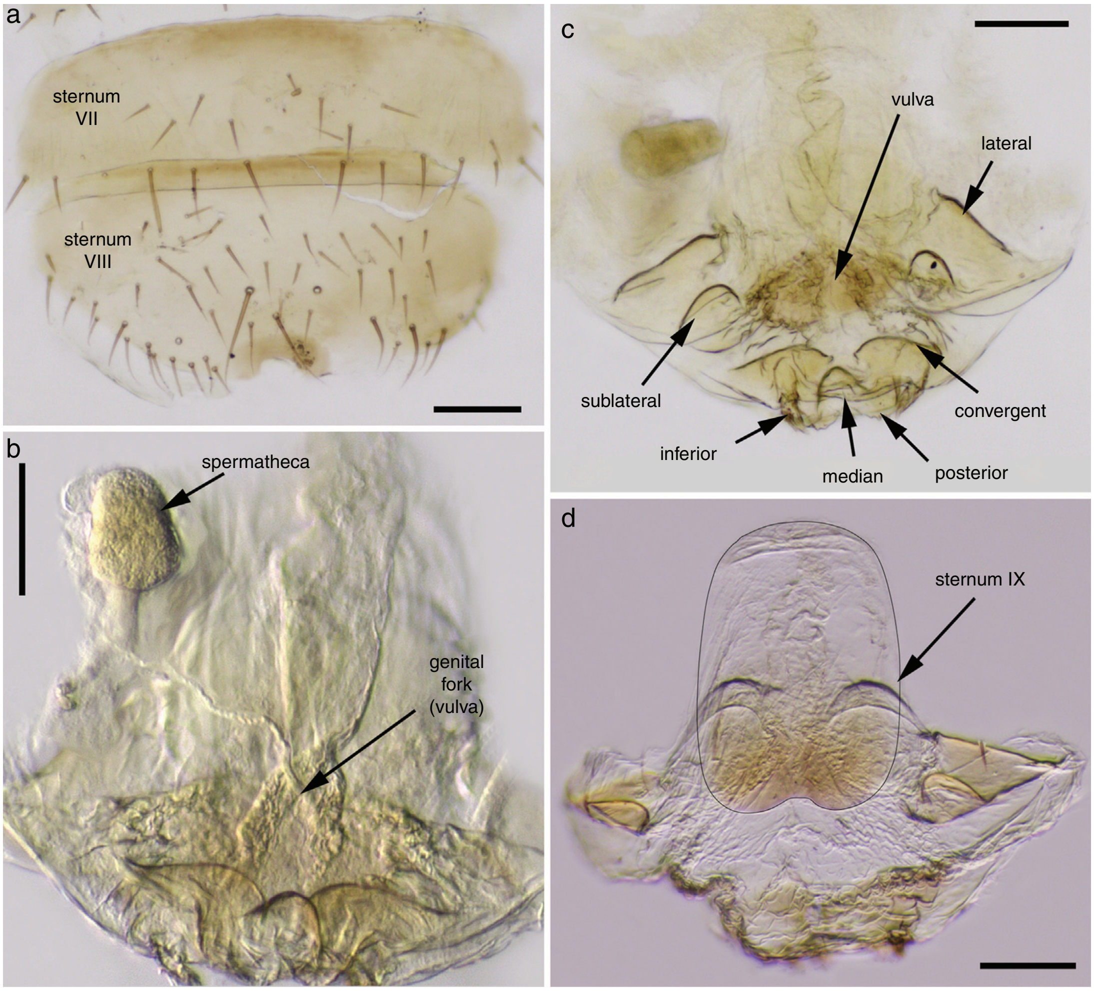

Fig. 3. Apical abdominal sclerites and genitalia of gynandromorph and normal-type female of *Zorotypus brasiliensis* Silvestri. (A) Ventral view of sternum VII and sternum VIII of gynandromorph. (B) Dorsal view of genitalia of gynandromorph, showing spermatheca, spermathecal duct, and genital fork (vulva). (C) Ventral view of genitalia of gynandromorph, with lobes labeled according Delamare Deboutteville (1956) and Silvestri (1947). (D) Dissected sternum IX, largely desclerotized, of normal-type female. *Scale* = 0.1 mm.

Wolbachia Hertig (Rickettsiaceae) is unknown but would be worthy of investigation given that temperature-sensitive suppression of *Wolbachia* elsewhere in insects can give rise to the expression of male traits. Thus, if the presence of *Wolbachia* is the cause of parthenogenesis in *Z. brasiliensis*, then its incidental suppression may be the reason for the discovery of the present gynandromorph. This might be easily tested from a reared lab colony whereby a subset of females are made to lay eggs at variously higher temperatures to see whether gynandromorphism could be artificially induced. It is therefore imperative to more fully study the biology of these populations in regard to their reproduction, potential bacterial associates, and, if truly parthenogenetic, whether there are different intranest ethologies resulting from the absence of males.

The gynandromorph itself is the first of its kind among more than 4000 zorapterans of various species sampled from Brazil. Naturally, the ultimate genetic cause of the gynandromorphism cannot be determined at this time, but the individual is best characterized as a bilateral gynandromorph (e.g., Dalla Torre and Friese, 1899; Wcislo et al., 2004; Michez et al., 2009; Campos et al., 2011; Hinojosa-Díaz et al., 2012). There are often few sexually dimorphic traits elsewhere on the body of zorapterans, although the males of some species have glandular orifices and distinctive setal patterns on the vertex. Thus, in the present gynandromorph it is not surprising that aside from the apical abdominal segments, no outwardly obvious features show sexual asymmetry. In fact, it was only the peculiar form of the apical terga that drew our initial attention.

When describing *Z. weidneri* from Amazonas, Brazil, New (1978) remarked on the similarities between females of his species and those of *Z. brasiliensis*. The gynandromorph described here gives some glimpse into some male traits that would be expressed presumably in normal-type males of *Z. brasiliensis*, and these, such as the arrangement of sensilla basiconica (see description, above). While the left half of the specimen does exhibit male features, and the epiprocts are not completely divided medially (reflecting the fact that the right half of the individual is female and therefore retains some medial fusion), it is interesting that there is little to no indication of the usual male genitalia within the genital chamber (see Delamare Deboutteville, 1956). Instead, the sclerites that can be discerned are degenerate forms of the usual arrangement of lobes and ducts associated with the female genitalia (*cf.* Fig. 3b and c with those of Silvestri, 1947). Externally, however, there is clearly a mating hook present (Fig. 2a and b), a feature of males. Given that the internal genitalia, while augmented from the normal-type female, are not evidently associated with male sclerites it is not possible to use these to easily homologize between the male and female systems of Zoraptera. Hopefully, continued exploration will reveal additional intersexes from which further information might be obtained.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgments

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